OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® HP Tissue DNA Midi Kit

D5197-00	2 preps
D5197-02	25 preps

May 2013

For research use only.Not intended for diagnostic testing.

E.Z.N.A.® HP Tissue DNA Midi Kit

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Introduction and Overview

The E.Z.N.A.® HP Tissue DNA Midi Kit is designed for efficient recovery of genomic DNA up to 60 kb in size from up to 500mg of tissue samples. The special designed buffer system ensure the optimal lysis of tissue rich in fat, polysaccharides, and fibers such as brain, adipose, and muscles. In addition, this kit can isolate DNA from molluscs, insects, arthropods, roundworms, flatworms, and other invertebrate tissue samples rich in mucopolysaccharides. The procedure relies on the well established properties of the cationic detergent, cetyltrimethyl ammonium bromide (CTAB), in conjunction with the selective DNA binding capabilities of Omega Bio-tek's HiBind® matrix.

Samples are homogenized and lysed in a high salt buffer containing CTAB and digested with proteinase. After addition of chloroform, the homogenate is separate into aqueous and organic phases by centrifugation. The upper, aqueous phase is extracted and BL Buffer and ethanol are added to provide appropriate binding conditions. The sample is transferred to the HiBind® DNA Midi Column, where the genomic DNA binds to the membrane and salt and other contaminants are efficiently washed way. High-quality genomic DNA is eluted with Elution Buffer or water. Purified DNA is suitable for most downstream applications such as endonuclease digestion, thermal cycle amplification, and hybridization techniques.

New in this Edition: This manual has been edited for content and redesigned to enhance user readability.

- Equilibration Buffer is no longer included with this kit. An optional Column Equilibration Protocol has been added to the protocol for your convenience.
- Equilibration Buffer is replaced with 3M NaOH provided by the user.
- HB Buffer has been replaced with HBC Buffer. HBC Buffer must be diluted with isopropanol (provided by user) before use.
- HB Buffer is no longer included with this kit.

Kit Contents

Product	D5197-00	D5197-02
Purifications	2	25
HiBind® DNA Midi Columns	2	25
15 mL Collection Tubes	2	25
MTL1 Buffer	8 mL	90 mL
BL Buffer*	8 mL	90 mL
HBC Buffer	5 mL	40 mL
Proteinase K Solution	250 µL	3 mL
RNase A	50 µL	550 µL
DNA Wash Buffer	5 mL	40 mL
Elution Buffer	5 mL	60 mL
User Manual	✓	✓

* BL Buffer contains a chaotropic salt. Use gloves and protective eye wear when handling this solution.

Storage and Stability

All of the E.Z.N.A.® HP Tissue DNA Midi Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. RNase A should be stored at 2-8˚C. Proteinase K Solution can be stored at room temperature for 12 months. For long-term storage (>12 months) store at 2-8°C. Store all other components at room temperature (22-25°C). Check Buffers for precipitates before use. Redissolve any precipitates by warming to 37˚C.

- Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D5197-00	20 mL
D5197-02	160 mL

- Dilute HBC Buffer with isopropanol as follows and store at room temperature.

Kit	Isopropanol to be Added
D5197-00	2 mL
D5197-02	16 mL

E.Z.N.A.® HP Tissue DNA Midi Kit Protocol

Materials and Equipment to be Supplied by User:

- Centrifuge equipped with swinging-bucket rotor capable of 2,000-5,000 × *g*
- Water baths, heat blocks, or incubators capable of 55-70°C
- Nuclease-free 15 mL centrifuge tubes
- 100% ethanol
- Isopropanol
- Sterile deionized water
- Chloroform
- Isoamyl alcohol

Before Starting:

- Prepare DNA Wash Buffer and HBC Buffer according to the directions in the "Preparing Reagents" section on Page 4.
- Prepare a mixture of chloroform:isoamyl alcohol (24:1).
- Heat water baths, heat blocks, or incubators capable of 55, 60, and 70°C.
- Heat Elution Buffer to 70°C.

1. Pulverize 200 mg tissue in liquid nitrogen with mortar and pestle. Samples can also be ground and homogenized by bead mill.

   Note: The amount of starting material depends on sample type and can be increased if acceptable results are obtained with the suggested 200 mg starting amount. Use no more than 500 mg tissue per HiBind® DNA Midi Column as DNA binding capacity (400 µg) may be exceeded. Difficult tissues may require starting with less than 200 mg tissue and a doubling of all buffer volumes to ensure adequate lysis.

2. Transfer the powder in a clean 15 mL centrifuge tube.

3. Add 3 mL MTL1 Buffer and 100 µL Proteinase K Solution. Vortex briefly to mix.

4. Incubate at 60°C for a minimum of 2 hours or until entire sample is solubilized.

   Note: Actual incubation time varies and depends on elasticity of tissue. Most samples require no more than 4 hours. Alternatively an overnight incubation at 55°C will produce adequate results.

5. Add 3 mL chloroform:isoamyl alcohol (24:1). Vortex to mix.

6. Centrifuge at 4,000 x *g* for 5 minutes.

7. Carefully transfer the upper aqueous phase to a clean 15 mL centrifuge tube. Avoid the milky interface containing contaminants and inhibitors. In most cases, around 2 mL upper phase can be transferred.

   Note: This step will remove most of the polysaccharides and proteins from the lysate and improve spin column performance downstream. If very little upper aqueous layer is present, add 1mL MTL1 Buffer into the lysate and vortex to mix. Repeat Steps 6-7 above. Continue to the Optional RNase treatment or Step 8 below.

OPTIONAL: Certain tissues such as liver have high levels of RNA which will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point. Add 20 µL RNase A (25 mg/mL, assuming a sample size of 200 mg) and incubate at room temperature for 5 minutes. Proceed to Step 8.

8. Add 1 volume BL Buffer. Vortex to mix.

   Note: For example if the total upper aqueous phase volume is 2 mL, add 2 mL BL Buffer.

9. Incubate at 70°C for 10 minutes. A wispy precipitate may form on addition of Buffer BL, but does not interfere with DNA recovery.

10. Add 1 volume 100% ethanol. Vortex for 30 seconds to mix thoroughly. If any precipitates are seen at this point, break up the precipitates by passing through a needle using a syringe.

    Note: For example if the total upper aqueous phase volume is 2 mL, add 2 mL 100% ethanol.

Optional Protocol for Column Equilibration

1. Add 1 mL 3M NaOH to the HiBind® DNA Midi Column.
2. Let sit for 4 minutes at room temperature.
3. Centrifuge at 4,000 x *g* for 3 minutes.
4. Discard the filtrate and reuse the Collection Tube.

11. Transfer the sample from Step 10, including any precipitates that may have formed, to the HiBind® DNA Midi Column.

12. Centrifuge at 4,000 x *g* for 5 minutes.

13. Discard the filtrate and reuse the collection tube.

14. Repeat Steps 11-13 until all the sample has been transferred to the column.

15. Add 3 mL HBC Buffer.

    Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 4 for instructions.

16. Centrifuge at 4,000 x *g* for 2 minutes.

17. Discard the filtrate and reuse the collection tube.

18. Add 3.5 mL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see Page 4 for instructions.

19. Centrifuge at 4,000 x *g* for 2 minutes.

20. Discard the filtrate and reuse the collection tube.

21. Repeat Steps 18-20 for a second DNA Wash Buffer wash step.

22. Centrifuge the empty HiBind® DNA Midi Column at maximum speed (<8,000 x *g)* for 10 minutes to dry the column.

    Note: This step is critical for removal of trace ethanol that may interfere with downstream applications**.**

23. Transfer the HiBind® DNA Midi Column to a clean 15 mL centrifuge tube.

24. Add 0.5-1 mL Elution Buffer or water heated to 70°C.

25. Let sit for 2-5 minutes at room temperature.

26. Centrifuge at 4,000 x *g* for 5 minutes.

27. Repeat Steps 24-26 for a second elution step.

28. Store eluted DNA at -20°C.

    Note: Typically a total of 200 µg DNA with absorbance ratio (A_{260}/A_{280}) of 1.7-1.9 can be obtained from 0.5 g animal tissue. Yields vary depending on source and quantity of starting material used.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
Clogged Column	Incomplete lysis	Increase incubation time with Buffer MTL1 / Proteinase K. An overnight incubation may be necessary. Centrifuge to remove any undigested particles.
	Sample too large	Do not use more than the recommended amount of starting material. For large samples, divide into multiple tubes.
	Incomplete homogenization	Pulverize starting material in liquid nitrogen as indicated in liquid nitrogen to obtain a fine powder.
Problem	Cause	Solution
Low DNA yield	Clogged column	See above
	Poor elution	Repeat elution or increase elution volume. Incubation of column at 70°C for 5 minutes prior to centrifugation may increase yields.
	Poor binding to column	Adjust volumes of BL Buffer and ethanol in proportion.
	Improper washing	DNA Wash Buffer must be diluted with 100% ethanol as specified on Page 4 before use.
		HBC Buffer must be diluted with isopropanol as specified on Page 4 before use.
Problem	Cause	Solution
Low A_{260}/A_{280} ratio	Extended centrifugation during elution step	Column resin may be present in eluate. Avoid higher centrifugation speeds than specified. The material can be removed from the eluate by centrifugation; it will not interfere with PCR or restriction digests.
	Poor cell lysis	Increase incubation time with MTL1 Buffer / Proteinase K. An overnight incubation may be necessary.
	Trace protein contaminants	Following Step 14, wash column with 1.5 mL BL Buffer + 1.5 mL 100% ethanol before proceeding to Step 15.

Troubleshooting Guide

Problem	Cause	Solution
No DNA eluted	Poor cell lysis	Increase incubation time with MTL1 Buffer / Proteinase K. An overnight incubation may be necessary.
	Incomplete homogenization	Pulverize starting material in liquid nitrogen as indicated to obtain a fine powder.
	Ethanol was not added to sample	Before applying DNA sample to column, add BL Buffer and 100% ethanol as indicated in Steps 8 and 10, Page 6.
	Ethanol was not added to DNA Wash Buffer	Dilute DNA Wash Buffer with the indicated volume of 100% ethanol prior to use.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
BL Buffer (100 mL)	PD062
DNA Wash Buffer (100 mL)	PS010
Elution Buffer (100 mL)	PDR048
Proteinase K Solution (2 mL)	AC115
Proteinase K Solution (10 mL)	AC116

Notes: